PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#6
JRP
9/30/95

In Re Application Of	)	
	)	Group Art Unit: TBA
David J. KYLE	)	
	)	Examiner: TBA
Serial No. 08/358,474	)	
	)	Atty Docket: 0311.48526
Filed: December 19, 1994	)	

For: MICROBIAL OIL MIXTURES AND USES THEREOF

INFORMATION DISCLOSURE STATEMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

In support of Applicant's Petition to Make Special, applicant submits herewith an analysis of what are believed to be relevant documents for the above-referenced application for use by the Examiner. Copies of these patents, articles and abstracts and an accompanying form PTO-1449 are enclosed for the convenience of the Examiner. Applicant does not concede that all of the enclosed documents are prior art to the subject application. It is believed no fee is required for consideration of this paper; however, the Commissioner is authorized to charge any necessary fee to deposit account no. 19-0733.

The subject application is a division of U.S. Serial No. 07/944,739, filed September 14, 1992, which is a continuation of 07/645,457, filed January 24, 1991. A European application (No. 92904388.3) corresponding to the present application has been filed, based on International application No. PCT/US92/00522, and 9 documents were identified as relevant to the patentability of the application in the International Search Report and the Supplemental European Search Report. Copies of the International Search Report and the Supplemental European Search

Report are attached for the convenience of the Examiner. A detailed discussion of the documents cited in these two Search Reports, as well as additional documents cited during prosecution of the parent of the present application and/or various foreign counterparts, is provided below. This discussion points out how the claimed subject matter is distinguishable over this information. Copies of all of the documents discussed below are enclosed along with a form PTO-1449 listing all of the documents and copies of the Declarations under 37 C.F.R. § 1.132 submitted in the parent application.

THE INFORMATION

The Invention

The present inventor has for the first time discovered how to obtain oil compositions containing fatty acid residues in the desired ratios by use of one or more *microbial* oils which are substantially free of eicosapentaenoic acid (EPA) as ingredients. Using one or more of these low EPA microbial oils, oil blends can be prepared having fatty acid ratios of polyunsaturated fatty acids (PUFAs) comparable to those in human breast milk, with the PUFAs in the form of fatty acid residues in triglycerides. Prior to the present invention, oil blends with this fatty acid composition had only been made by hydrolyzing fish oil, purifying the resultant free fatty acids, and mixing the free fatty acids in the desired ratio. The product of such a process is a mixture of free fatty acids, not a triglyceride blend. The present claims exclude these prior art mixtures, by requiring that the PUFAs (specifically the ω-3 fatty acid docosahexaenoic acid or DHA and the ω-6 fatty acid arachidonic acid or ARA) be in triglyceride form and that at least one oil source be microbial.

Microbial Oils

The present inventor has discovered microbial sources of oil containing desired fatty acids (ARA and DHA) in the form of triglycerides. No oil sources disclosed in the cited art provide DHA or ARA in triglyceride form substantially free of EPA, other than the microbial sources identified by the inventors and disclosed in U.S. Patent No. 5,397,591 and International application No. PCT/US92/00517 (WO 92/13086). Triglycerides containing the desired fatty acid residues in the appropriate ratios (i.e., low EPA) can be readily obtained by extraction from the microbial source without need for further purification, in contrast to the prior art (which teaches fatty acid purification by hydrolysis under harsh conditions followed by fractionation of individual fatty acids). Thus, the blended oil of the present invention, containing at least one microbial oil with at least one of the desired fatty acids in triglyceride form, is novel, and the prior art does not suggest methods for obtaining this useful product, which were first disclosed by the present inventor.

Japanese patent application 01/215,245 (Suntory), appears to teach use of fungal biomass containing the appropriate fatty acids as an ingredient in various products. Therefore, isolated oil according to the present invention, and products containing the isolated oil rather than biomass or free fatty acids, are clearly different from the products described in the Japanese '245 application and do not appear to be suggested from the text of the application.

Suntory application JP 01/196,255 attempts production of infant formula which matches the fatty acid profile of human milk. This document also discusses the manufacture of infant formula by adding either fatty acids, or oils and fats containing these fatty acids, in the appropriate ratios to produce a product having the same amounts of the individual fatty acids as

human milk. The '255 application teaches preparation of one ingredient for the infant formula by culturing *Mortierella* microorganisms, recovering the biomass and extracting it with an organic solvent to obtain a lipid fraction. This lipid fraction can then be added as one of the ingredients in the preparation of infant formula. The '255 application does not specifically disclose high ARA/low EPA oil. It also does not provide DHA-containing oil low in EPA or GLA containing oil low in EPA.

In a series of papers, Shinmen, et al., and Totani, et al., reported studies on lipid metabolism of *Mortierella* sp. Various strains of *Mortierella* were grown and the lipids were analyzed for ARA (and sometimes EPA) as a percent of total fatty acid content (see Shinmen, et al. (1989), *Appl. Microb. Biotech.*, **31**:11-16; U.S. Pat. No. 5,204,250; EP 0 223 960; and EP 0 276 982; Yamada, et al. (1987), Proc. World Congr. Biotechnol. for Oils and Fats Industr., Applewhite, ed., Am. Oil Chem. Soc., p. 173; Totani, et al. (1987), *Lipids*, **22**:1060; Shimizu, et al. (1988), *J. Am. Oil Chem. Soc.*, **65**:1455; Totani, et al. (1988), in ISF-JOCS World Conference; Shimizu, et al. (1988), in ISF-JOCS World Conference; Yamada, et al. (1989), *J. Disp. Sci. & Tech.*, 10:561-579; and Shimizu, et al. (1989), *Appl. Microb. Biotechnol.*, **31**:1-4). In these documents, it is indicated that under various fermentation conditions, *Mortierella* strains will produce cellular lipid having ARA as up to 55% of the total lipid, with varying amounts of EPA. Yamada, et al. (1987) reported that ARA, EPA and another highly unsaturated fatty acid, bishomo-γ-linolenic acid, were found to accumulate in the polar lipids associated with the mycelial membrane, rather than in triglycerides (see Abstract and the sentence spanning columns 1 and 2 of page 175). Thus, the prior art did not suggest that

fungal oil would be a good source of triglycerides enriched in ARA, but substantially free of EPA.

JP 01/038,007 (Lion Corp.) describes a cosmetic preparation containing a lipid material from *Mortierella sp.* which is enriched in ARA. However, there is no indication of how much ARA is present or how much EPA is present. Indeed, fish oil is disclosed to be equivalent, suggesting that the presence of EPA is contemplated.

EP 0 223 960 (Lion) only describes production of ARA-containing oil, with no indication whether the ARA will be in triglyceride form or not. In contrast, the present application claims an oil blend in which ARA residues are in *triglyceride* form. Thus, the present process, which not only requires oil high in ARA, but requires the ARA in a particular form, is novel over EP 0223 960.

JP 01/304 892 (Suntory, Ltd.) describes producing microbes enriched in polyunsaturated fatty acids, but there is no indication of what form the fatty acid residues are in, nor what the relative amounts of various fatty acid residues are. In particular, JP '892 does not describe microbial oil containing at least 10% ARA and substantially free of EPA.

U.S. Patent Nos. 4,874,629 (Chang, et al) and 4,963,385 (Antrim, et al), deal with purification and stabilization of high omega-3 oils, without any suggestion of enriching with DHA. The claimed oils and products cannot be obtained in such a manner.

Long (International Patent Publication WO89/00606 which corresponds to Australian application 21226/88) teaches shake flask culture of a variety of fungi and algae to produce omega-3 fatty acids in low yield. Long, is a prophetic publication which suggests culturing marine microorganisms to produce lipid products containing omega-3 fatty acids, but does not

indicate whether the omega-3 fatty acids will be in the form of triglycerides nor what proportion of the fatty acids may be DHA or EPA. The disclosure of Long describes fermentation of microbes to produce ω-3 fatty acids which are taught to be both EPA and DHA; there is no indication of how to separate the fatty acid species or how to obtain them in any particular form. Long does not teach or suggest conditions that will result in production of high levels of DHA-containing triglyceride oil by dinoflagellates. Thus, the high DHA triglyceride oil used in the present invention is not provided by Long.

Ratledge (1986, in Baldwin, et al., eds., *Proc. World Conf. Emerging Technol. Fats Oils Industr.*, Am. Oil Chem Soc., pp. 318-330) indicates that conditions which increase lipid content of the biomass (nitrogen limitation) will also decrease biomass yield. Beach, et al., discussed below, also teach that *Crypthecodinium cohnii* may only be grown at low biomass levels (3 g/L), and that conditions which increase oil will decrease biomass. Most algae produce starch or other carbohydrate as storage material under metabolic limitation such as low nitrogen. Only relatively few algae produce lipid as a storage material, and Ratledge includes a review of the literature on these lipid-producing algae.

Ratledge indicates that nitrogen limitation is well known to increase the lipid content of lipid-producing algae generally, noting that the lipid content can be elevated to as much as 85% of the biomass. However, Ratledge indicates that conditions which increase lipid content of the biomass (nitrogen limitation) will also decrease biomass yield. Thus, the general statement in Ratledge (based on the teaching of underlying references) that nitrogen limitation will increase lipid production in algae does not provide a commercial source for oil production in general or for DHA production in particular.

Neither Ratledge nor any of the underlying articles disclose the DHA content of algal triglycerides nor do they suggest the use of dinoflagellates for DHA production. These underlying references indicate that the lipid-producing algae are not generally considered to include the Dinophyceae, particularly not heterotrophic Dinophyceae such as *Crypthecodinium*. In reviewing these underlying references, Ratledge does not suggest the use of dinoflagellates for oil production nor disclose the DHA content of algal triglycerides produced by any algal species. Ratledge does provide a list of algae which are suggested for use in oil production (Table 2, p. 321), and the list includes algae from the classes Chlorophyceae, Rhodophyceae, and Euglenophyceae, but no dinoflagellates.

Ratledge does not discuss the heterotrophic dinoflagellates, nor do the references cited in Ratledge, especially Aaronson, et al., Shifrin, et al. (1980); and Shifrin (1984). Aaronson, et al., 1980 (in Algal Biomass, Shelef, et al., eds., Elsevier, pp. 575-601, cited on page 319 of Ratledge), lists "lipid" content for a large number of microalgae, including 15 cyanobacteria, 7 diatoms, 28 Chlorophyceae, 4 Haptophyceae, 4 Phaeophyceae, 4 Rodophyceae, 4 Dinophyceae,[1] 3 Xanthophyceae, and 2 Prasinophyceae, but there is no indication that the "lipid" contains any fatty acyl material, let alone triglycerides, or that DHA in any form is produced by any of these organisms. Shifrin and Chisholm, 1980 (in Algal Biomass, Shelef, et al., eds., Elsevier, pp. 627-645, cited on page 319 of Ratledge), discuss only Clorophyceae and diatoms. Shifrin, et al. (1980) does not disclose heterotrophic Dinophyceae. Shifrin, 1984 (in Biotechnology for the Oils and Fats Industry, Ratledge, et al., eds., American Oil Chemists Society, Champaign, IL, pp. 145-162, cited on page 319 of Ratledge), lists 51 algal species in

[1]The Dinophyceae listed in Aaronson, et al., are all phototrophic.

Table 2 and 18 algal species in Table 3, including 58 Chlorophyceae, 36 diatoms, 23 cyanobacteria, 3 macroalgae, 4 Chrysophceae, 3 Haptophyceae, 2 Rhodophyceae, 1 Cryptophyceae, and 1 Prochlorophyceae, but no Dinophyceae, but there is no indication that the "lipid" contains any fatty acyl material, let alone triglycerides, or that DHA in any form is produced by any of these organisms. The underlying references cited by Ratledge indicate that the lipid-producing algae are not generally considered to include the Dinophyceae, particularly not heterotrophic Dinophyceae.

Yongmanitchai, et al. (*Process Biochem.*, 24:117-125, 1989), Volkman, et al. (1989, "Fatty Acid and Lipid Composition of 10 Species of Microalgae Used in Mariculture," *J. Exp. Mar. Biol. Ecol.*, 128:219-240), and Ben-Amotz. (1985, "Chemical Profile of Selected Species of Microalgae With Emphasis on Lipids," *J. Phycol.*, 21:72-81), report on the content of polyunsaturated fatty acids as a percent of total fatty acids for a variety of microorganisms. However, there is no indication whether these polyunsaturated acids appear primarily in the phospholipid form or some other form in these organisms (Ben-Amotz reports neutral and polar lipid contents, but not the fatty acid content of these fractions). Indeed, the need for polyunsaturated fatty acids in phospholipids in order to maintain membrane fluidity and proper biological functioning is well-known. Therefore, these documents do not provide the skilled worker with a source of DHA in triglyceride form.

Pohl, et al. (1979, in Hoppe, et al., eds., *Marine Algae in Pharmaceutical Science*, de Gruyter, Berlin, pp. 473-523) is merely a review article that does not provide guidance on how to produce oil of any particular composition. Pohl, et al., provides no guidance whatever concerning conditions for fermentation which would produce oil having properties recited in the

present claims. Furthermore, while Pohl, et al., discloses that *C. cohnii* lipids may contain up to 30% DHA with no eicosapentaenoic acid (EPA), and also that the lipids are made up of primarily triglycerides and phospholipids, there is no indication in Pohl, et al., of how the DHA is distributed between triglycerides and phospholipids. Thus, the art does not suggest that a triglyceride oil containing a high level DHA can be obtained from *C. cohnii*. Indeed, Pohl combined with Harrington and Henderson (discussed below) would suggest that lipid extracted from *C. cohnii* would **not** contain triglycerides having at least about 20% DHA.

Fermenting heterotrophic dinoflagellates to high biomass levels containing high DHA triglycerides requires high levels of nutrients as well as vigorous oxygenation to keep up with the oxygen demand of the biomass. These conditions, leading to high biomass levels, were first taught in the inventor's application which ripened into U.S. Patent No. 5,407,957 (first published August 22, 1991, as WO 91/11918). Claim 1 indicates that the process according to this invention includes a step of recovering triglyceride oil (in contrast to lipid extracts containing significant amounts of polar material). Pohl, et al., does not teach or suggest conditions that will result in production of high levels of DHA-containing triglyceride oil by dinoflagellates, or indeed, that dinoflagellates can produce triglycerides containing high amounts of DHA. Thus, the claimed triglyceride oil and methods of producing it are not obvious in view of Pohl.

Beach, et al. (1973, *Biochim. Biophys. Acta*, **316**:56-65), discloses that *Crypthecodinium cohnii* can produce lipids with greater than 40% DHA content, but Beach et al., indicates that most of the DHA will be in the form of phospholipids (see Table 1 and Figure 1). This is consistent with Harrington, et al. (*J. Protozool.*, 17:213-219, 1970), which indicates that 90%

of the DHA is in the phospholipid fraction (Table 6) and with Henderson, et al. (*Phytochem.*, 27:1679-1683, 1988), who report up to 57% DHA in phospholipid, but less than 7% DHA in triglycerides from *C. cohnii*. Thus, Beach, et al. does not suggest that *C. cohnii* are a source of triglyceride oil containing high amounts of DHA.

In order to achieve a lipid fraction with a relatively high content of DHA, the cells must be cultured in the presence of air, but increased aeration is taught by Beach, et al., to decrease the total and neutral lipid yield of the culture. In either shake flasks or fermentors, Beach, et al., would not lead the ordinary worker to reasonably expect production of a lipid fraction with triglyceride component high in DHA by *C. cohnii*.

Beach et al., produce oil very low in triglycerides, which means that extensive purification is required before a material which is high in triglycerides can be obtained. Such extensive purification is both expensive and likely to reduce the quality of the oil as a result of, e.g., oxidation of highly unsaturated fatty acids, such as DHA, during the multiple purification steps. The present invention relies on microbial production of edible oil which is already high in triglycerides containing DHA and/or ARA. Microbial oils which are already high in triglycerides containing PUFAs can be extracted and blended to form the claimed oil *product* that is distinct from blends made using a triglyceride fraction purified from the lipid extract taught in Beach, et al. (in which any desirable minor components will have been removed from the oil along with the contaminants by more extensive processing).

Sonnenborn, et al., (*Biochem. Biophys. Acta*, 712:523-534, 1982) deals with a single enzyme found within *C. cohnii*: fatty acid synthase. This enzyme is involved in fatty acid synthesis, but is not the enzyme that determines whether the fatty acid will be DHA, ARA or

any other PUFA. Thus, Sonnenborn, et al., is not relevant to claims reciting the DHA content of oils or oil blends and does not suggest a source of oil having PUFAs present in triglyceride form. The teaching of Sonnenborn, et al., would provide no guidance to skilled worker interested in developing a method for production of triglycerides containing PUFAs.

Barclay, Australian specification 67195/90 (corresponds to WO 91/07498 and U.S. Patent No. 5,130,242) provides no indication concerning the form in which omega-3 fatty acids are found in the *Thraustochytriales*. Indeed, the preferred method for recovering omega-3 fatty acids taught in Barclay requires hydrolysis *in situ* to produce free fatty acids which are then recovered and fractionated to produce high omega-3 fatty acid compositions. The claims of Barclay are directed to omega-3 fatty acids and to biomass containing them. They do not specify the particular fatty acids nor the form in which they are produced. Thus, claims to triglyceride oil, in which the omega-3 fatty acid DHA makes up at least 20% of the fatty acid residues in the triglycerides, is not disclosed or claimed By Barclay.

Gamma-Linolenic Acid Oils

Claims which are drawn to blends of DHA-enriched microbial oils and ARA or gamma-linoleic acid (GLA) enriched oil are novel and non-obvious over Traitler, et al (U.S. Patent No. 4,938,984) or Tillott (Austr. Appln. No. 44856/89, corresponding to WO 90/04391) and Ingenbleek, et al., (U.S. Patent No. 4,526,793). Traitler, et al., teaches a method of obtaining triglycerides containing gamma-linolenic acid from plant material, and any oil blends containing the gamma-linolenic acid are made from animal, plant or mineral oils. There is no suggestion to use microbes as a source of oil for any oil blends. Traitler or Tillott (Austr. Appln. No. 44856/89, corresponding to WO 90/04391) do not describe any high DHA/low EPA fatty acid

sources, and the only oil exemplified is fish oil, which provides high EPA/low DHA oil instead. Ingenbleek does not teach the use of any fatty acids with more than 18 carbons.

Herbert, U.S. Patent No. 4,851,343, EP 269 351 (Lion), Hanada, JP 01/132,371, and Hansson, et al. (1988, "Effect of Culture Conditions on Mycelial Growth and Production of gamma-Linolenic Acid by the Fungus *Mortierella ramanniana*," *Appl. Microbiol. Biotechnol.*, 28:240-246), disclose production of gamma-linolenic acid using microbial sources, but do not indicate whether or not the gamma-linolenic acid is found in triglycerides. Further, there is no suggestion that the gamma-linolenic acid obtained according to the teaching of either EP 269 351 or Hanada is suitable for use in food or in nutrient supplements or infant formula, as claimed in the present application.

Infant Formula

Clandinin, et al., U.S. Patent No. 4,670,285, and "Long Chain Fatty Acids in Human Milk: Are They of Benefit to the Newborn?" in *Composition and Physiological Properties of Human Milk*, Proc. of the Internat'l. Workshop on the Composition of Physiological Properties of Human Milk, Kiel, Germany, Schaub, J., ed., 1985, supply a target ratio of fatty acids for infant formula but do not enable preparation of a composition meeting the target ratios with triglyceride oils containing these fatty acids, because the necessary ingredients were not available to Clandinin. Indeed, the sources that Clandinin, et al., list have either lipids containing equal amounts of DHA and EPA (e.g., fish oils) or lipids in which DHA is in the phospholipid fraction (e.g., egg yolk). Clandinin, et al., do not suggest that low EPA triglyceride oils would be desirable, nor that the DHA or EPA should be in the triglyceride form. Clandinin identifies

egg yolk as a source of C_{22} acids, but the C_{22} acids from this source are in the form of phospholipids.

The present claims are directed to infant formula and baby food containing *triglyceride* oil in which the triglycerides are high in DHA and/or ARA and low in EPA. The only triglyceride oil sources disclosed by Clandinin, et al., are animal (especially marine) and plant oils. Clandinin, et al. ('285), does not teach or suggest a source of triglycerides which are high in DHA and low in EPA. There is no suggestion of a microbial source for the desired fatty acids. Clandinin, et al., cannot teach or suggest infant formula or baby food containing high DHA triglyceride oils that were not available to Clandinin, et al., in the art. In the absence of known sources for triglyceride oils high in DHA and without any significant quantity of EPA, Clandinin, et al., would not enable the ordinary worker to produce the claimed products.

The fatty acid composition of human milk is also discussed in Bracco, et al., 1978, "Human Milk Lipids and Problems Related To Their Replacement," extract from *Annales Nestle, No. 40*, 55-81; Bitman, et al., 1983, "Comparison of the Lipid Composition of Breast Milk from Mothers of Term and Preterm Infants," *Amer. J. Clin. Nutr.*, 38:300-312; and Harzer, et al., 1983, "Changing Patterns of Human Milk Lipids in the Course of the Lactation and During the Day," *Am. J. Clin. Nutr.*, 37:612-621.

U.S. Pat. No. 5,013,569 (Rubin), discloses infant formula supplemented with free acids of both EPA and docosahexaenoic acid, but not supplemented with ARA. The claimed infant formula which is high in ARA but low in EPA cannot be anticipated by infant formula of Rubin which is high in EPA, not low.

Cotter, et al., (U.S. Pat. No. 4,920,098) is concerned with nutritional supplements for human nutrition and infant formula, and teaches the need to include essential fatty acids in the compositions for these end uses. However, the only oil sources disclosed are animal (especially marine) and plant oils. There is no suggestion of a microbial source for the desired fatty acids.

U.S. Patent Nos. 4,868,001 (Maruta); 4,960,795 (Salte, et al.); 4,911,944 (Holub); JP 02/257,835 (Tagen Chem.); CA 100:66638d (Kame, et al., 1984); and Liu, et al. (1987, "Increase in Plasma Phospholipid Docosahexaenoic and Eicosapentaenoic Acids as a Reflection of Their Intake and Mode of Administration," *Pedatr. Res.*, 22:292-296); describe the supplementation of human food or animal feed with fatty mixtures containing omega-3 fatty acids, and the only source exemplified is fish oil.

Innis, et al. (*Am. J. of Clin. Nutri.*, 1990, 51:994-1000), recommends supplementing infant formula with C18 fatty acids, but not with DHA (a C22 fatty acid).

EP 0 140 805 describes infant formula which contains unsaturated fatty acids extracted from placenta. This does not suggest the microbial source disclosed by Applicants. The preferred source is, in fact, the phospholipid fraction of the placental extract, teaching away from the triglyceride oil of the present invention.

El Boustani, et al. (1987, "Enteral Absorption in Man of Eicosapentaenoic Acid in Different Chemical Forms," *Lipids*, 22:711-714), teaches that ethyl esters of EPA are absorbed more slowly than the free fatty acid form, thereby also teaching away from the present invention.

EP 0 148 303 is concerned with dietary supplements having a high content of polyunsaturated fatty acids, which may include ARA. There is no indication how much ARA

is present, and the disclosure is silent concerning EPA. Clearly, this does not suggest the compositions of this invention blended from oil which contains at least 10% ARA and is substantially free of EPA.

Schweikhardt, et al., (EP 231 904, corresponding to DE 3603000) teaches an oil-containing product having nearly equal amounts of DHA and EPA in the total fatty acid composition (page 7). Schweikhardt, et al., does not address the issue of what form the fatty acids are in, nor does it teach or suggest a source of triglycerides which are high in DHA and low in EPA. Schweikhardt, et al., does not teach or suggest infant formula or baby food containing high DHA triglyceride oils that were not available in the art.

Therapeutic Fatty Acids

Rubin, U.S. Patent No. 4,526,902, issued 07/88; Horrobin, U.S. Patent No. 4,681,896, issued 07/87; Rubin, et al., U.S. Patent No. 4,792,418, issued 12/88; Horrobin, U.S. Patent No. 4,810,497, issued 3/89; Stewart, et al., U.S. Patent No. 4,826,877, issued 05/89; Horrobin, et al., U.S. Patent No. 5,116,871, issued 05/92; Horrobin, U.S. Patent No. 5,120,760, issued 06/92; Horrobin, U.S. Patent No. 5,198,468, issued 03/93; and EP 296 751, published 12/88 (Efamol Holdings); discuss the medical consequences of low tissue levels of polyunsaturated fatty acids including ARA, DHA and EPA, and recommend increasing the level of these fatty acid in particular patients, but the only sources taught for these fatty acids are animal (marine) oils and plant oils. Where particular fatty acids are recommended, the only method taught to obtain the necessary material is hydrolysis to the level of free fatty acids, followed by fractionation and recovery of the desire pure fatty acid.

The following patents and articles were cited in the parent applications. Applicant believes that these documents are merely cumulative over the documents discussed above, so the following documents are not further discussed. However, they are listed here for completeness.

U.S. Patents

Bernhart, et al., U.S. Patent No. 2,6 11,706, issued 09/52;

Otto, U.S. Patent No. 2,923,628, issued 02/60;

Ensor, et al., U.S. Patent No. 3,458,625, issued 07/69;

Tomarelli, et al., U.S. Patent No. 3,542,560, issued 11/70;

Bernhart, et al., U.S. Patent No. 3,649,295, issued 03/72;

Williams, U.S. Patent No. 4,058,594, issued 11/77;

Mueller, et al., U.S. Patent No. 4,216,236, issued 08/80;

Theuer, U.S. Patent No. 4,282,265, issued 08/81;

Gaull, U.S. Patent No. 4,303,692, issued 12/81;

Recivi, et al., U.S. Patent No. 4,513,008, issued 04/85;

Gil, et al., U.S. Patent No. 4,544,559, issued 10/85;

Rule, U.S. Patent No. 4,614,663, issued 09/86;

Mascioli, et al., U.S. Patent No. 4,752,618, issued 06/88;

Pistolesi, et al., U.S. Patent No. 4,780,456, issued 10/88;

Mascioli, et al., U.S. Patent No. D 4,820,731, issued 04/89;

Rubin, U.S. Patent No. 4,843,095, issued 06/89;

Fratzer, U.S. Patent No. 4,874,603, issued 10/89;

King, et al., U.S. Patent No. 4,876,107, issued 10/89;

Katz, et al., U.S. Patent No. 5,234,702, issued 08/93;

Thepenier, et al., U.S. Patent No. 5,338,673, issued 08/94.

Foreign Patents

JP 60/160 840, published 12/83 (Miyoshi Oil and Fat) (abst. only);

JP 64/080 250, published 3/89 (Snow);

WO 90/13656, published 11/90 (Enzytech);

EP 404 058, published 12/90 (Milupa);

Articles

Sanders, et al., 1978, "Studies of Vegans: The Fatty Acid Composition of Plasma Choline Phosphoglycerides, Erythrocytes, Adipose Tissue, and Breast Milk, and some Indicators of Susceptibility to Ischemic Heart Disease in Vegans and Omnivore Controls," *Am. J. Clin. Nutri.*, 31:805-813

Carlson, 1987, "Effect of Fish Oil Supplementation on the n-3 Fatty Acid Content of Red Blood Cell Membranes in Preterm Infants," *Pediatri. Res.*, 21:507-510 (Abst. Only)

Puppione, et al., 1988, "Marine Mammals" Animal Models for Studying the Digestion and Transport of Dietary Fats Enriched in ω-3 Fatty Acids. Positional Analyses of Milk Fat Triacylglycerol Molecules," in *Dietary ω-3 and ω-6 Fatty Acids*, Galli, et al., eds., Plenum Press

Weaver, et al., 1989, "The Effect of Positional Placement of EPA in Ingested Triglyceride on EPA Accumulation in Human Platelet and Plasma Phospholipids," in *Health Effects of Fish And Fish Oils*, Clandra, ed., St. John's, Newfoundland

Ackman, R.G., 1989, "Problems in Fish Oils and Concentrates," in *Fats For The Future*, Chap. 13, pp. 189-200, Cambie, ed.

Yeh, et al., 1990, "Enrichment of (n-3) Fatty Acids of Suckling Rats by Maternal Dietary Menhaden Oil," *J. Nutr.*, 120

Carlson, et al., 1990, "Growth and Development of Very Low-Birthweight Infants in Relation to n-3 and n-6 Essential Fatty Acid Status," *Inform*, 1:192-196 (Essential Fatty Acids and Eicosanoids -- Invited Papers from the Third International Congress, American Oil Chemists' Society, Champaign Ill.

Bourre, et al., 1990, "Desaturase in Brain and Liver During Development and Aging," *Lipids*, 25:354-356

Simopoules, 1990, in *Omega-3 Fatty Acids in Health And Disease*, Lees, et al., eds., p. 136

Kyle, 1991, *Adv. Applied Biotech.*, 12

Carlson, et al., 1992, *Essential Fatty Acids And Eicosenoids*, Sinclair, et al., eds., American Oil Chem. Society, Champaign, Illinois

In summary, the blended oil of the present invention, containing at least one microbial oil with at least one of the desired fatty acids in triglyceride form, is novel, and no combination of documents provided herein teach or suggest methods for obtaining this useful product, which were first disclosed by the present inventors. Claims in the present application directed to oil blends consisting of ARA- and/or DHA-enriched microbial oils diluted with a third oil with or

without ARA, DHA or EPA are novel and non-obvious over the prior art to the same extent as an oil blend consisting only of two microbial oils.

The present claims require oil blends having DHA and ARA in triglyceride form and that the level of EPA be reduced relative to the ARA/DHA level. Such blends were not taught or suggested in the art prior to Applicant's disclosure. The present inventor first disclosed that triglycerides containing the desired fatty acid residues in the appropriate ratios can be readily obtained by extraction from the microbial source without need for further purification, in contrast to the prior art (which teaches enrichment of fatty acids by hydrolysis of source oil under harsh conditions followed by fractionation of individual fatty acids). The claims of the present application exclude mixtures of fatty acids in accordance with the prior art, by requiring that the PUFAs (DHA and ARA, in particular) be in triglyceride form and that at least one oil source be microbial.

The Examiner is respectfully requested to consider the above documents, and make them of record in the application.

Respectfully submitted,

Date: June 28, 1995

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